

SEQUENCE LISTING

<110> Novozymes A/S

Svendsen, Allan

Andersen, Carsten

Borchert, Torben Vedel

<120> Pullulanase variants and methods for preparing such variants with predetermined properties

<130> 6072.204-US

<140> To Be Assigned

<141> 2001-08-21

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<170> PatentIn version 3.1

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gcg act gag cat cca atg gca caa aag ttt gtg ctt gat tca gtt aat Ala Thr Glu His Pro Met Ala Gln Lys Phe Val Leu Asp Ser Val Asn 595 600 605			1824
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gct ctt tta gga aaa gac acg atg gca aaa ata tca aac gag ctg cat Ala Leu Leu Gly Lys Asp Thr Met Ala Lys Ile Ser Asn Glu Leu His 625 630 635 640			1920
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Gln Phe Asp Trp Ser Arg Lys Ala Gln Phe Lys Asp Val Phe Asp Tyr	805	810	815	
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Phe Ser Ser Met Ile His Leu Arg Asn Gln His Pro Ala Phe Arg Met	820	825	830	
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Thr Thr Ala Asp Gln Ile Lys Gln Asn Leu Thr Phe Leu Glu Ser Pro	835	840	845	
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Thr Asn Thr Val Ala Phe Glu Leu Lys Asn Tyr Ala Asn His Asp Thr	850	855	860	
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Trp Lys Asn Ile Ile Val Met Tyr Asn Pro Asn Lys Thr Ser Gln Thr	865	870	875	880
ctt aat cta cca agt gga gat tgg acc att gta gga ttg gga gat caa				2688
Leu Asn Leu Pro Ser Gly Asp Trp Thr Ile Val Gly Leu Gly Asp Gln	885	890	895	
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Ile Gly Glu Lys Ser Leu Gly His Val Met Gly Asn Val Gln Val Pro	900	905	910	
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Ala Ile Ser Thr Leu Ile Leu Lys Gln	915	920		

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<213> Bacillus acidopullulyticus

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Ala Val Ala Asp Thr Gln Val Pro Gly Asp Asn Thr Gln Val Gly Leu
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Ile Val Arg Lys Asn Asp Trp Ser Glu Lys Asn Thr Pro Asn Asp Leu
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Pro Thr Ile Tyr	Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ala Ile Pro					
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Ser Val Ser	Asn Ala Tyr Leu Asp Asp Glu Lys Thr Val Leu Ala Lys					
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Val Tyr Ser Lys Asp	Ala Thr Ser Phe Arg Val Trp Ala Pro Thr Ala					

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Gln	Phe	Asp	Trp	Ser	Arg	Lys	Ala	Gln	Phe	Lys	Asp	Val	Phe	Asp	Tyr		
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Leu Asn Leu Pro Ser Gly Asp Trp Thr Ile Val Gly Leu Gly Asp Gln
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gtc Val	aat Asn 610	gag Glu	tat Tyr	cat His	att Ile	gac Asp 615	ggc Gly	ttc Phe	cgt Arg	ttt Phe	gac Asp 620	tta Leu	atg Met	gcg Ala	ctg Leu	1872
ctt Leu 625	gga Gly	aaa Lys	gac Asp	acg Thr	atg Met 630	tcc Ser	aaa Lys	gct Ala	gcc Ala	tcg Ser 635	gag Glu	ctt Leu	cat His	gct Ala	att Ile 640	1920
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gtc Val 690	ttt Phe	gat Asp	tct Ser	tcc Ser	gct Ala	caa Gln 695	ggg Gly	ttt Phe	gcg Ala	aca Thr	ggg Gly 700	gca Ala	aca Thr	ggc Gly	tta Leu	2112
act Thr 705	gat Asp	gca Ala	att Ile	aag Lys	aat Asn 710	ggc Gly	gtt Val	gag Glu	ggg Gly 715	agt Ser	att Ile	aat Asn	gac Asp	ttt Phe 720	acc Thr	2160
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gat Asp	cgg Arg	att Ile 755	aaa Lys	atg Met	gat Asp	gaa Glu	ctc Leu 760	gca Ala	caa Gln	gca Ala	gtt Val 765	gtt Val	atg Met	acc Thr	tca Ser	2304
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gat Asp	tgg Trp	agc Ser	agg Arg	aaa Lys 805	gct Ala	caa Gln	tat Tyr	cca Pro	gat Asp 810	gtt Val	ttc Phe	aac Asn	tat Tyr	tat Tyr 815	agc Ser	2448
ggg Gly	cta Leu	atc Ile	cac His 820	ctt Leu	cgt Arg	ctt Leu	gat Asp	cac His 825	cca Pro	gcc Ala	ttc Phe	cgc Arg	atg Met 830	acg Thr	aca Thr	2496
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aca gtg gcc tat gaa tta act gat cat gtt aat aaa gac aaa tgg gga Thr Val Ala Tyr Glu Leu Thr Asp His Val Asn Lys Asp Lys Trp Gly 850 855 860	2592
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Ala Glu Tyr Asp Phe Asn Gln Pro Ala Asp Ser Phe Gly Ala Val Ala 35 40 45
Ser Ala Asp Ile Pro Gly Asn Pro Ser Gln Val Gly Ile Ile Val Arg 50 55 60
Thr Gln Asp Trp Thr Lys Asp Val Ser Ala Asp Arg Tyr Ile Asp Leu 65 70 75 80
Ser Lys Gly Asn Glu Val Trp Leu Val Glu Gly Asn Ser Gln Ile Phe 85 90 95
Tyr Asn Glu Lys Asp Ala Glu Asp Ala Ala Lys Pro Ala Val Ser Asn 100 105 110
Ala Tyr Leu Asp Ala Ser Asn Gln Val Leu Val Lys Leu Ser Gln Pro 113

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Ala	Asn	Lys	Asp	Ile	Pro	Val	Thr	Ser	Val	Lys	Asp	Ala	Ser	Leu	Gly
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Gln	Asp	Val	Thr	Ala	Val	Leu	Ala	Gly	Thr	Phe	Gln	His	Ile	Phe	Gly
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Gly	Ser	Asp	Trp	Ala	Pro	Asp	Asn	His	Ser	Thr	Leu	Leu	Lys	Lys	Val
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Thr	Asn	Asn	Leu	Tyr	Gln	Phe	Ser	Gly	Asp	Leu	Pro	Glu	Gly	Asn	Tyr
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Phe	Ser	Tyr	Ile	Pro	Ser	Thr	His	Ala	Val	Tyr	Asp	Thr	Ile	Asn	Asn
				245					250					255	
Pro	Asn	Ala	Asp	Leu	Gln	Val	Glu	Ser	Gly	Val	Lys	Thr	Asp	Leu	Val
			260					265					270		
Thr	Val	Thr	Leu	Gly	Glu	Asp	Pro	Asp	Val	Ser	His	Thr	Leu	Ser	Ile
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Gln	Thr	Asp	Gly	Tyr	Gln	Ala	Lys	Gln	Val	Ile	Pro	Arg	Asn	Val	Leu
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Thr	Gln	Lys	Ala	Thr	Thr	Phe	Lys	Val	Trp	Ala	Pro	Thr	Ser	Thr	Gln
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Val	Pro	Met	Thr	Ala	Ser	Gly	His	Gly	Val	Trp	Glu	Ala	Thr	Val	Asn
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370

375

380

Ser Thr Arg Thr Ala Val Asp Pro Tyr Ala Thr Ala Ile Ala Pro Asn
 385 390 395 400

Gly Thr Arg Gly Met Ile Val Asp Leu Ala Lys Thr Asp Pro Ala Gly
 405 410 415

Trp Asn Ser Asp Lys His Ile Thr Pro Lys Asn Ile Glu Asp Glu Val
 420 425 430

Ile Tyr Glu Met Asp Val Arg Asp Phe Ser Ile Asp Pro Asn Ser Gly
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Met Lys Asn Lys Gly Lys Tyr Leu Ala Leu Thr Glu Lys Gly Thr Lys
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Gly Pro Asp Asn Val Lys Thr Gly Ile Asp Ser Leu Lys Gln Leu Gly
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Ile Thr His Val Gln Leu Met Pro Val Phe Ala Ser Asn Ser Val Asp
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Glu Thr Asp Pro Thr Gln Asp Asn Trp Gly Tyr Asp Pro Arg Asn Tyr
 500 505 510

Asp Val Pro Glu Gly Gln Tyr Ala Thr Asn Ala Asn Gly Asn Ala Arg
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Ile Lys Glu Phe Lys Glu Met Val Leu Ser Leu His Arg Glu His Ile
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Gly Val Asn Met Asp Val Val Tyr Asn His Thr Phe Ala Thr Gln Ile
 545 550 555 560

Ser Asp Phe Asp Lys Ile Val Pro Glu Tyr Tyr Tyr Arg Thr Asp Asp
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Ala Gly Asn Tyr Thr Asn Gly Ser Gly Thr Gly Asn Glu Ile Ala Ala
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Glu Arg Pro Met Val Gln Lys Phe Ile Ile Asp Ser Leu Lys Tyr Trp
 595 600 605

Val Asn Glu Tyr His Ile Asp Gly Phe Arg Phe Asp Leu Met Ala Leu
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Leu Gly Lys Asp Thr Met Ser Lys Ala Ala Ser Glu Leu His Ala Ile
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Ser	Ser	Pro	Gly	Glu	Thr	Ile	Asn	Tyr	Val	Thr	Ser	His	Asp	Asn	Tyr
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Thr	Leu	Trp	Asp	Lys	Ile	Ala	Leu	Ser	Asn	Pro	Asn	Asp	Ser	Glu	Ala
			740					745					750		
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Gln	Gly	Val	Pro	Phe	Met	Gln	Gly	Gly	Glu	Glu	Met	Leu	Arg	Thr	Lys
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Gly	Gly	Asn	Asp	Asn	Ser	Tyr	Asn	Ala	Gly	Asp	Ala	Val	Asn	Glu	Phe
785					790					795					800
Asp	Trp	Ser	Arg	Lys	Ala	Gln	Tyr	Pro	Asp	Val	Phe	Asn	Tyr	Tyr	Ser
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	850					855					860				
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885

890

895

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 Asn Tyr Ala Asn Trp Asp Leu Trp Met Trp Pro Tyr Gln Pro Val Asn
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ggt aat gga gca gca tac gag ttt tct gga aag gat gat ttt ggc gtt 144
 Gly Asn Gly Ala Ala Tyr Glu Phe Ser Gly Lys Asp Asp Phe Gly Val
 35 40 45

aaa gca gat gtt caa gtg cct ggg gat gat aca cag gta ggt ctg att 192
 Lys Ala Asp Val Gln Val Pro Gly Asp Asp Thr Gln Val Gly Leu Ile
 50 55 60

gtc cgt aca aat gat tgg agc caa aaa aat aca tca gac gat ctc cat 240
 Val Arg Thr Asn Asp Trp Ser Gln Lys Asn Thr Ser Asp Asp Leu His
 65 70 75 80

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 Ile Asp Leu Thr Lys Gly His Glu Ile Trp Ile Val Gln Gly Asp Pro
 85 90 95

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 Asn Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ala Thr Pro Lys
 100 105 110

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 Val Ser Asn Ala Tyr Leu Asp Asn Glu Lys Thr Val Leu Ala Lys Leu
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aaa Lys	aat Asn 610	aga Arg	gtt Val	atg Met	gga Gly	agt Ser 615	att Ile	tca Ser	gat Asp	ttc Phe	act Thr 620	tcg Ser	gca Ala	cct Pro	agc Ser	1872
gaa Glu 625	acc Thr	att Ile	aac Asn	tat Tyr	gta Val 630	aca Thr	agc Ser	cat His	gat Asp	aat Asn 635	atg Met	aca Thr	ttg Leu	tgg Trp	gat Asp 640	1920

aaa att agc gca agt aat ccg aac gat aca caa gca gat cga att aag Lys Ile Ser Ala Ser Asn Pro Asn Asp Thr Gln Ala Asp Arg Ile Lys	1968
att gat gaa ttg gct caa gct gtg gta ttt act tca caa ggg gta cca Met Asp Glu Leu Ala Gln Ala Val Val Phe Thr Ser Gln Gly Val Pro	2016
ttt atg caa ggt gga gaa gaa atg ctg cgg aca aaa ggc ggt aat gat Phe Met Gln Gly Gly Glu Glu Met Leu Arg Thr Lys Gly Gly Asn Asp	2064
aat agt tac aat gcc ggg gat agc gtg aat cag ttc gat tgg tca aga Asn Ser Tyr Asn Ala Gly Asp Ser Val Asn Gln Phe Asp Trp Ser Arg	2112
aaa gca caa ttt gaa aat gta ttc gac tac tat tct tgg ttg att cat Lys Ala Gln Phe Glu Asn Val Phe Asp Tyr Tyr Ser Trp Leu Ile His	2160
cta cgt gat aat cac cca gca ttc cgt atg acg aca gcg gat caa atc Leu Arg Asp Asn His Pro Ala Phe Arg Met Thr Thr Ala Asp Gln Ile	2208
aaa caa aat ctc act ttc ttg gat agc cca acg aac act gta gca ttt Lys Gln Asn Leu Thr Phe Leu Asp Ser Pro Thr Asn Thr Val Ala Phe	2256
gaa tta aaa aat cat gcc aat cat gat aaa tgg aaa aac att ata gtt Glu Leu Lys Asn His Ala Asn His Asp Lys Trp Lys Asn Ile Ile Val	2304
atg tat aat cca aat aaa act gca caa act ctc act cta cca agt gga Met Tyr Asn Pro Asn Lys Thr Ala Gln Thr Leu Thr Leu Pro Ser Gly	2352
aat tgg aca att gta gga tta ggc aat caa gta ggt gag aaa tca cta Asn Trp Thr Ile Val Gly Leu Gly Asn Gln Val Gly Glu Lys Ser Leu	2400
ggc cat gta aat ggc acg gtt gag gtg cca gct ctt agt acg atc att Gly His Val Asn Gly Thr Val Glu Val Pro Ala Leu Ser Thr Ile Ile	2448
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 20 25 30
 Gly Asn Gly Ala Ala Tyr Glu Phe Ser Gly Lys Asp Asp Phe Gly Val
 35 40 45
 Lys Ala Asp Val Gln Val Pro Gly Asp Asp Thr Gln Val Gly Leu Ile
 50 55 60
 Val Arg Thr Asn Asp Trp Ser Gln Lys Asn Thr Ser Asp Asp Leu His
 65 70 75 80
 Ile Asp Leu Thr Lys Gly His Glu Ile Trp Ile Val Gln Gly Asp Pro
 85 90 95
 Asn Ile Tyr Tyr Asn Leu Ser Asp Ala Gln Ala Ala Ala Thr Pro Lys
 100 105 110
 Val Ser Asn Ala Tyr Leu Asp Asn Glu Lys Thr Val Leu Ala Lys Leu
 115 120 125
 Thr Asn Pro Met Thr Leu Ser Asp Gly Ser Ser Gly Phe Thr Val Thr
 130 135 140
 Asp Lys Thr Thr Gly Glu Gln Ile Pro Val Thr Ala Ala Thr Asn Ala
 145 150 155 160
 Asn Ser Ala Ser Ser Ser Glu Gln Thr Asp Leu Val Gln Leu Thr Leu
 165 170 175
 Ala Ser Ala Pro Asp Val Ser His Thr Ile Gln Val Gly Ala Ala Gly
 180 185 190
 Tyr Glu Ala Val Asn Leu Ile Pro Arg Asn Val Leu Asn Leu Pro Arg
 195 200 205
 Tyr Tyr Tyr Ser Gly Asn Asp Leu Gly Asn Val Tyr Ser Asn Lys Ala
 210 215 220
 Thr Ala Phe Arg Val Trp Ala Pro Thr Ala Ser Asp Val Gln Leu Leu
 225 230 235 240
 Leu Tyr Asn Ser Glu Thr Gly Pro Val Thr Lys Gln Leu Glu Met Gln
 245 250 255
 Lys Ser Asp Asn Gly Thr Trp Lys Leu Lys Val Pro Gly Asn Leu Lys
 260 265 270

Asn Trp Tyr Tyr Leu Tyr Gln Val Thr Val Asn Gly Lys Thr Gln Thr
 275 280 285

Ala Val Asp Pro Tyr Val Arg Ala Ile Ser Val Asn Ala Thr Arg Gly
 290 295 300

Met Ile Val Asp Leu Glu Asp Thr Asn Pro Pro Gly Trp Lys Glu Asp
 305 310 315 320

His Gln Gln Thr Pro Ala Asn Pro Val Asp Glu Val Ile Tyr Glu Val
 325 330 335

His Val Arg Asp Phe Ser Ile Asp Ala Asn Ser Gly Met Lys Asn Lys
 340 345 350

Gly Lys Tyr Leu Ala Phe Thr Glu His Gly Thr Lys Gly Pro Asp Asn
 355 360 365

Val Lys Thr Gly Ile Asp Ser Leu Lys Glu Leu Gly Ile Asn Ala Val
 370 375 380

Gln Leu Gln Pro Ile Glu Glu Phe Asn Ser Ile Asp Glu Thr Gln Pro
 385 390 395 400

Asn Met Tyr Asn Trp Gly Tyr Asp Pro Arg Asn Tyr Asn Val Pro Glu
 405 410 415

Gly Ala Tyr Ala Thr Thr Pro Glu Gly Thr Ala Arg Ile Thr Gln Leu
 420 425 430

Lys Gln Leu Ile Gln Ser Ile His Lys Asp Arg Ile Ala Ile Asn Met
 435 440 445

Asp Val Val Tyr Asn His Thr Phe Asn Val Gly Val Ser Asp Phe Asp
 450 455 460

Lys Ile Val Pro Gln Tyr Tyr Tyr Arg Thr Asp Ser Ala Gly Asn Tyr
 465 470 475 480

Thr Asn Gly Ser Gly Val Gly Asn Glu Ile Ala Thr Glu Arg Pro Met
 485 490 495

Val Gln Lys Phe Val Leu Asp Ser Val Lys Tyr Trp Val Lys Glu Tyr
 500 505 510

His Ile Asp Gly Phe Arg Phe Asp Leu Met Ala Leu Leu Gly Lys Asp
 515 520 525

Thr Met Ala Lys Ile Ser Lys Glu Leu His Ala Ile Asn Pro Gly Ile
530 535 540

Val Leu Tyr Gly Glu Pro Trp Thr Gly Gly Thr Ser Gly Leu Ser Ser
545 550 555 560

Asp Gln Leu Val Thr Lys Gly Gln Gln Lys Gly Leu Gly Ile Gly Val
565 570 575

Phe Asn Asp Asn Ile Arg Asn Gly Leu Asp Gly Asn Val Phe Asp Lys
580 585 590

Ser Ala Gln Gly Phe Ala Thr Gly Asp Pro Asn Gln Val Asn Val Ile
595 600 605

Lys Asn Arg Val Met Gly Ser Ile Ser Asp Phe Thr Ser Ala Pro Ser
610 615 620

Glu Thr Ile Asn Tyr Val Thr Ser His Asp Asn Met Thr Leu Trp Asp
625 630 635 640

Lys Ile Ser Ala Ser Asn Pro Asn Asp Thr Gln Ala Asp Arg Ile Lys
645 650 655

Met Asp Glu Leu Ala Gln Ala Val Val Phe Thr Ser Gln Gly Val Pro
660 665 670

Phe Met Gln Gly Gly Glu Glu Met Leu Arg Thr Lys Gly Gly Asn Asp
675 680 685

Asn Ser Tyr Asn Ala Gly Asp Ser Val Asn Gln Phe Asp Trp Ser Arg
690 695 700

Lys Ala Gln Phe Glu Asn Val Phe Asp Tyr Tyr Ser Trp Leu Ile His
705 710 715 720

Leu Arg Asp Asn His Pro Ala Phe Arg Met Thr Thr Ala Asp Gln Ile
725 730 735

Lys Gln Asn Leu Thr Phe Leu Asp Ser Pro Thr Asn Thr Val Ala Phe
740 745 750

Glu Leu Lys Asn His Ala Asn His Asp Lys Trp Lys Asn Ile Ile Val
755 760 765

Met Tyr Asn Pro Asn Lys Thr Ala Gln Thr Leu Thr Leu Pro Ser Gly
770 775 780

Asn Trp Thr Ile Val Gly Leu Gly Asn Gln Val Gly Glu Lys Ser Leu
785 790 795 800

Gly His Val Asn Gly Thr Val Glu Val Pro Ala Leu Ser Thr Ile Ile
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Leu His Gln Gly Thr Ser Glu Asp Val Ile Asp Gln Asn
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